KM(1)

29N1

#30NM

PATENT
Attorney Docket No. 2356.0074

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Agnes LABIGNE et al.	Group Art Unit: 1645
	Examiner: N. Minnifield
Serial No.: 08/211,312	
Filed: July 1, 1994	NOTICE OF ALLOWANCE DATED: 10/27/98
For: GENES OF HELICOBACTER PYLORI NECESSARY FOR THE REGULATION AND MATURATION OF UREASE AND THEIR USE (AS AMENDED)	Batch No.: K86

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

RECEIVED
Publishing Division
JAN 05 1999

SUBMISSION OF FORMAL DRAWINGS

Subject to the approval of the Examiner, please replace the informal drawings with the formal drawings Fig. 1 - 13 (20 sheets) filed herewith. If the formal drawings for any reason are not in full compliance with the pertinent statutes and regulations, please so advise the undersigned. If any fees are necessary for the submission of these formal drawings, please charge our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

By: Leslie McDonell
Leslie A. McDonell
Reg. No. 34,872

Dated: January 5, 1999

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08/211312

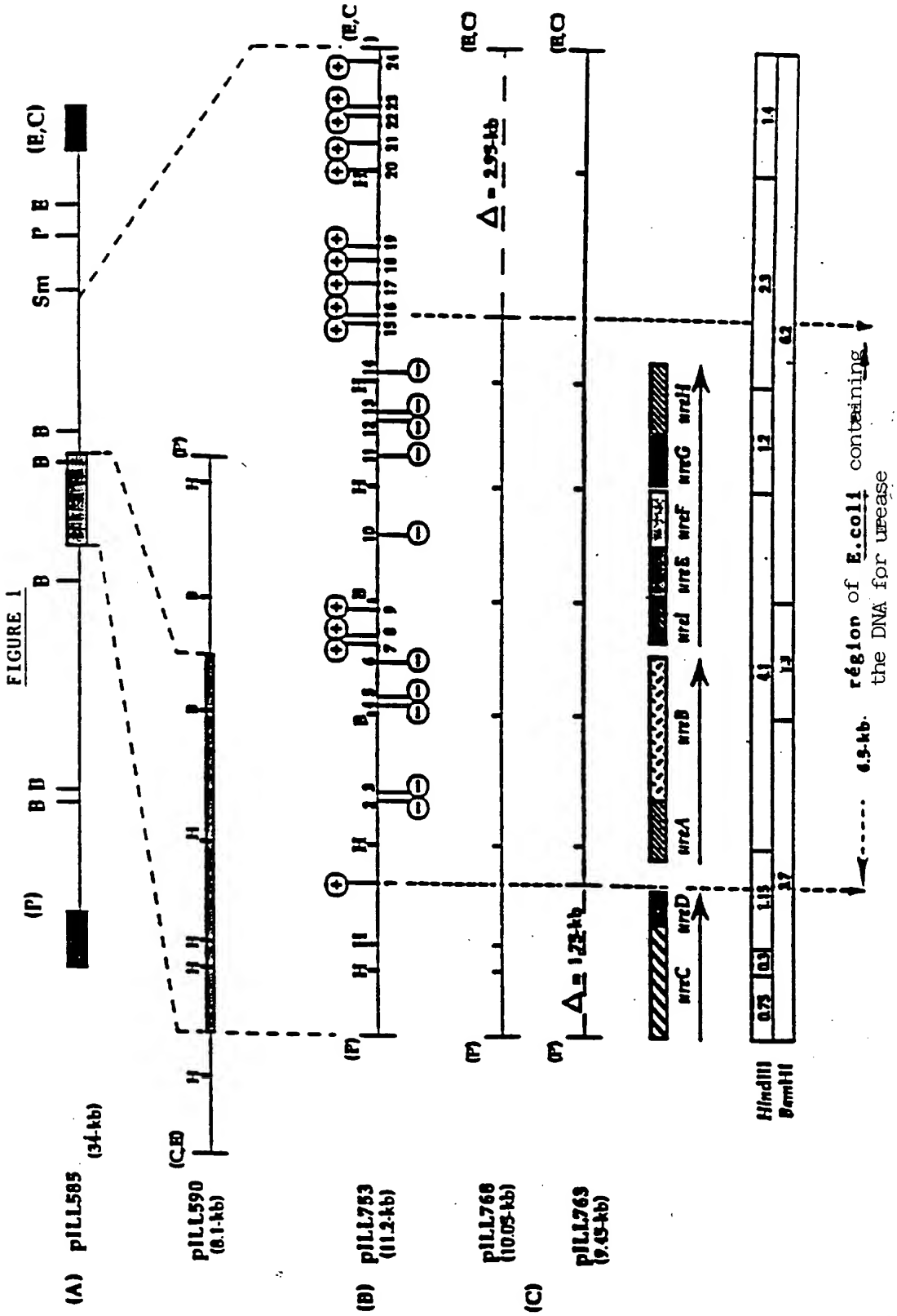

FIGURE 1

région of E.coli containing the DNA for uPease

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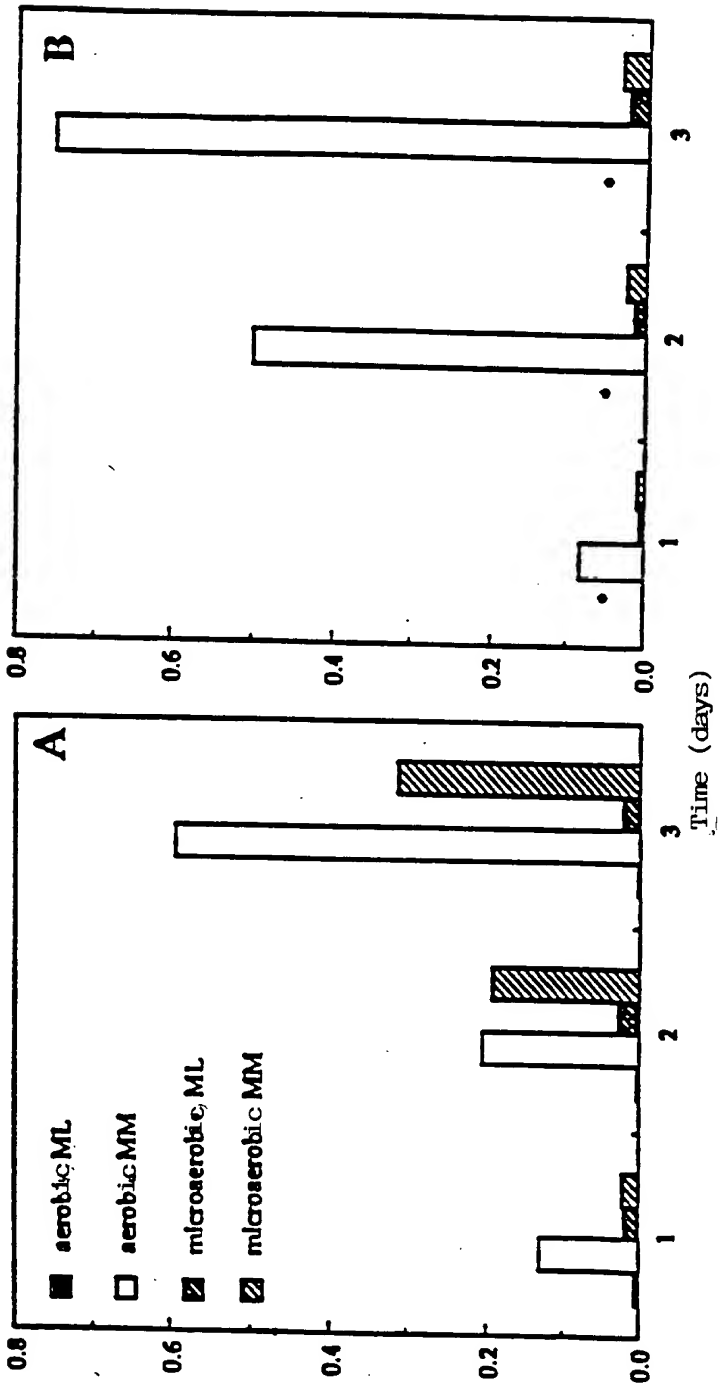
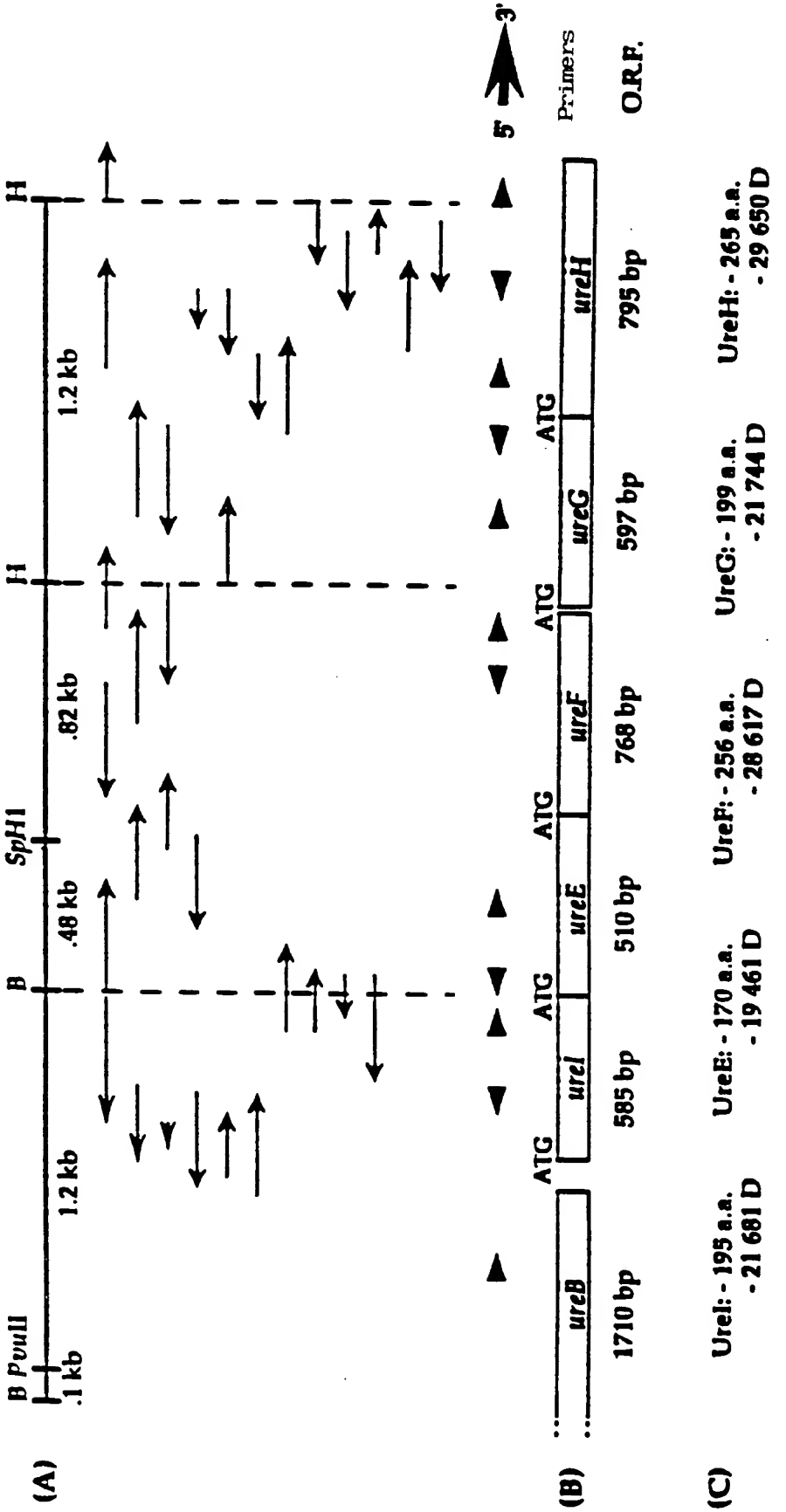

B
A
aerobic ML
aerobic MM
microaerobic ML
microaerobic MM
0.8
0.6
0.4
0.2
0.0
1
2
3
Time (days)
Urease activity
(umol urea/min./mg protein

FIGURE 2


(A)
B PvuII
.1 kb
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B
.48 kb
SpHI
.82 kb
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1.2 kb
H
(B)
5'
3'
Primers
ureB
ATG
1710 bp
ureI
ATG
585 bp
ureE
ATG
510 bp
ureF
ATG
768 bp
ureG
ATG
597 bp
ureH
795 bp
(C)
O.R.F.
UreI: - 195 a.a.
- 21 681 D
UreE: - 170 a.a.
- 19 461 D
UreF: - 256 a.a.
- 28 617 D
UreG: - 199 a.a.
- 21 744 D
UreH: - 265 a.a.
- 29 650 D

FIGURE 3

FIGURE 4

```
1                                                   31
A CTC TTT AGC ATT TTC TAG GA TTT TTT AGG AGC AAC GCT CTT AGA TCC TTA GTT TTT AGC
--leu phe ser ile phe AMB
61                                                  91
TCT CTG ATT TTT TGT TTA TCA AAA AAT TGG GGG CTT TTT TTG TTT TTA TTT TTT GTC AAT

121                                                 151
TTA CTA TTT TTC TTT ATG ATT AGC TCA AGC AAC AAA AGT TAT TCG TAA GGT GCG TTT GTT

181                      SD                         211
GTA AAA ATT TTT GTT TGG AAG GAA AAG GCA ATG CTA GGA CTT GTA TTG TTA TAT GTT GGG
         ureI                                       Met leu gly leu val leu leu tyr val gly

241                                                 271
ATT GTT TTA ATC AGC AAT GGG ATT TGC GGG TTA ACC AAA GTC GAT CCT AAA AGC ACT GCG
ile val leu ile ser asn gly ile cys gly leu thr lys val asp pro lys ser thr ala

301                                                 331
GTG ATG AAC TTT TTT GTG GGT GGG CTC TCC ATT ATT TGT AAT GTG GTT GTC ATC ACT TAT
val met asn phe phe val gly gly leu ser ile ile cys asn val val val ile thr tyr

361                                                 391
TCC GCT CTC AAC CCT ACA GCC CCT GTA GAA GGT GCT GAA GAT ATT GCT CAA GTA TCA CAC
ser ala leu asn pro thr ala pro val glu gly ala glu asp ile ala gln val ser his

421                                                 451
CAT TTG ACT AAT TTC TAT GGG CCA GCG ACT GGG TTA TTG TTT GGT TTC ACC TAC TTG TAT
his leu thr asn phe tyr gly pro ala thr gly leu leu phe gly phe thr tyr leu tyr
```

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FIGURE 4 (cont.)

```
481                                                 511
GCG GCT ATC AAC CAC ACT TTT GGT TTG GAT TGG AGG CCC TAC TCT TGG TAT AGC TTA TTC
ala ala ile asn his thr phe gly leu asp trp arg pro tyr ser trp tyr ser leu phe

541                                                 571
GTA GCG ATC AAC ACG ATT CCT GCT GCG ATT TTA TCC CAC TAT AGC GAT ATG CTT GAT GAC
val ala ile asn thr ile pro ala ala ile leu ser his tyr ser asp met leu asp asp

601                                                 631
CAC AAA GTG TTA GGC ATC ACT GAA GGC GAT TGG TGG GCG ATC ATT TGG TTG GCT TGG GGT
his lys val leu gly ile thr glu gly asp trp trp ala ile ile trp leu ala trp gly

661                                                 691
GTT TTG TGG CTT ACC GCT TTC ATT GAA AAC ATC TTG AAA ATC CCT TTA GGG AAA TTC ACT
val leu trp leu thr ala phe ile glu asn ile leu lys ile pro leu gly lys phe thr

721                                                 751
CCA TGG CTT GCT ATC ATT GAG GGC ATT TTA ACC GCT TGG ATC CCT GCT TGG TTA CTC TTT
pro trp leu ala ile ile glu gly ile leu thr ala trp ile pro ala trp leu leu phe

781                                                 811
ATC CAA CAC TGG GTG TGA GAT GAT CAT
ile gln his trp val OPA

782                                                 812
TCC AAC ACT GGG TGT GAG ATG ATC ATA GAG CGT TTA ATA GGC AAT CTA AGG GAT TTA AAC
            ureE      Met ile ile glu arg leu ile gly asn leu arg asp leu asn
```

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FIGURE 4 (cont.)

842 871
CCC TTG GAT TTC AGC GTG GAT TAT GTG GAT TTG GAA TGG TTT GAA ACG AGG AAA AAA ATC
pro leu asp phe ser val asp tyr val asp leu glu trp phe glu thr arg lys lys ile

902 932
GCT CGC TTT AAA ACC AGG CAA GGC AAA GAC ATA GCC GTA CGC CTT AAA GAC GCT CCC AAG
ala arg phe lys thr arg gln gly lys asp ile ala val arg leu lys asp ala pro lys

962 992
TTG GGT TTC TCT CAA GGA GAT ATT TTA TTT AAA GAA GAG AAG GAA ATT ATC GCC GTT AAT
leu gly phe ser gln gly asp ile leu phe lys glu glu lys glu ile ile ala val asn

1022 1052
ATC TTG GAT TCT GAA GTC ATT CAC ATC CAA GCT AAG AGC GTG GCA GAA GTA GCG AAA ATA
ile leu asp ser glu val ile his ile gln ala lys ser val ala glu val ala lys ile

1082 1112
TGC TAT GAA ATA GGA AAC CGC CAT GCG GCT TTA TAC TAT GGC GAG TCT CAA TTT GAA TTT
cys tyr glu ile gly asn arg his ala ala leu tyr tyr gly glu ser gln phe glu phe

1142 1172
AAA ACA CCA TTT GAA AAG CCC ACG CTA GCG TTA CTA GAA AAG CTA GGG GTT CAA AAT CGT
lys thr pro phe glu lys pro thr leu ala leu leu glu lys leu gly val gln asn arg

1202 1232
GTT TTA AGT TCA AAA TTG GAT TCC AAA GAA CGC TTA ACC GTG AGC ATG CCC CAT AGT GAG
val leu ser ser lys leu asp ser lys glu arg leu thr val ser met pro his ser glu

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FIGURE 4 (cont.)

```
1262                                        1292                    SD
CCT AAT TTT AAG GTC TCA CTG GCG AGC GAT TTT AAA GTG GTC ATG AAA TAG AAA AAC AA
pro asn phe lys val ser leu ala ser asp phe lys val val met lys AMB

1321                                        1351
CAA ATG GAT AAA GGA AAA AGC GTG AAA AGC ATT GAA AAA AGC GTG GGT ATG CTC CCA AAA
F   Met asp lys gly lys ser val lys ser ile glu lys ser val gly met leu pro lys

1381                                        1411
ACT CCA AAG ACA GAC AGC AAT GCT CAT GTG GAT AAT GAA TTT CTG ATT CTG CAA GTC AAT
thr pro lys thr asp ser asn ala his val asp asn glu phe leu ile leu gln val asn

1441                                        1471
GAT GCG GTG TTC CCC ATT GGA TCT TAC ACG CAT TCT TTT GGG CTT TTG GCT AGA AAC TTA
asp ala val phe pro ile gly ser tyr thr his ser phe gly leu leu ala arg asn leu

1501                                        1531
CAT CCA GCA AAA AAG GTT ACT AAT AAA GAA AGC GCT TTA AAA TAT TTA AAA GCC AAT CTC
his pro ala lys lys val thr asn lys glu ser ala leu lys tyr leu lys ala asn leu

1561                                        1591
TCT AGC CAG TTC CTT TAC ACG GAA ATG CTG AGC TTG AAA CTC ACC TAT GAA AGC GCT CTC
ser ser gln phe leu tyr thr glu met leu ser leu lys leu thr tyr glu ser ala leu

1621                                        1651
CAA CAA GAT TTA AAA AGG ATC TTA GGG GTT GAA GAA ATC ATT ACG CTA TCC ACA AGC CCC
gln gln asp leu lys arg ile leu gly val glu glu ile ile thr leu ser thr ser pro
```


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PCT/FR92/00921

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FIGURE 4 (cont.)

```
1681                                                    1711
ATG GAA TTG CGA TTA GCC AAT CAA AAG CTA GGC AAT CGT TTC ATT AAA ACC TTA CAA GCC
met glu leu arg leu ala asn gln lys leu gly asn arg phe ile lys thr leu gln ala

1741                                                    1771
ATG AAC GAA TTA GAC ATT GGC GCA TTT TTT AAC GCT TAC GCT CAA CAA ACC GAA GAC CCC
met asn glu leu asp ile gly ala phe phe asn ala tyr ala gln gln thr glu asp pro

1801                                                    1831
ACC CAT GCC ACT AGC TAT GGC GTT TTT GCG GCG AGT TTG GGG ATT GAA TTG AAA AAG GCT
thr his ala thr ser tyr gly val phe ala ala ser leu gly ile glu leu lys lys ala

1861                                                    1891
TTA AGG CAT TAT CTT TAT GCA CAA ACT TCT AAC ATG GTA ATT AAC TGC GTT AAA AGC GTC
leu arg his tyr leu tyr ala gln thr ser asn met val ile asn cys val lys ser val

1921                                                    1951
CCA CTA TCT CAA AAC GAT GGG CAA AAA ATC TTA TTG AGC TTG CAA AGC CCT TTT AAC CAG
pro leu ser gln asn asp gly gln lys ile leu leu ser leu gln ser pro phe asn gln

1981                                                    2011
CTC ATA GAA AAA ACC CTA GAA CTA GAC GAA AGC CAC TTG TGC GCG GCA AGC GTT CAA AAC
leu ile glu lys thr leu glu leu asp glu ser his leu cys ala ala ser val gln asn

2041                                                    2071
GAC ATT AAG GCG ATG CAG CAT GAG AGT TTA TAC TCG CGC CTT TAT ATG TCT TGA ATT TTA
asp ile lys ala met gln his glu ser leu tyr ser arg leu tyr met ser OPA
```

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FIGURE 4 (cont.)

```
2102          SD                                  2132
TCT CAA ATT GAA AGG AAT TTT ATG GTA AAA ATT GGA GTT TGT GGT CCT GTA GGA AGC GGT
   ureG                        Met val lys ile gly val cys gly pro val gly ser gly
2162                                              2192
AAA ACC GCC TTG ATT GAA GCT TTA ACG CGC CAC ATG TCA AAA GAT TAT GAC ATG GCG GTC
lys thr ala leu ile glu ala leu thr arg his met ser lys asp tyr asp met ala val

2222                                              2252
ATC ACT AAT GAT ATT TAC ACG AAA GAA GAC GCA GAA TTT ATG TGT AAA AAT TCG GTG ATG
ile thr asn asp ile tyr thr lys glu asp ala glu phe met cys lys asn ser val met

2282                                              2312
CCA CGA GAG AGG ATC ATT GGC GTA GAA ACA GGA GGC TGT CCG CAC ACG GCT ATT AGA GAA
pro arg glu arg ile ile gly val glu thr gly gly cys pro his thr ala ile arg glu

2342                                              2372
GAC GCT TCT ATG AAT TTA GAA GCC GTA GAA GAA ATG CAT GGC CGT TTC CCT AAT TTG GAA
asp ala ser met asn leu glu ala val glu glu met his gly arg phe pro asn leu glu

2402                                              2432
TTG CTT TTG ATT GAA AGC GGA GGC AGT AAC CTT TCA GCG ACT TTC AAC CCA GAG CTA GCG
leu leu leu ile glu ser gly gly ser asn leu ser ala thr phe asn pro glu leu ala

2462                                              2492
GAC TTT ACG ATC TTT GTG ATT GAT GTG GCT GAG GGC GAT AAA ATC CCC AGA AAA GGC GGG
asp phe thr ile phe val ile asp val ala glu gly asp lys ile pro arg lys gly gly
```

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FIGURE 4 (cont.)

```
2522                                                    2552
CCA GGA ATC ACG CGT TCA GAC TTG CTT GTC ATC AAT AAG ATT GAT TTA GCC CCC TAT GTG
pro gly ile thr arg ser asp leu leu val ile asn lys ile asp leu ala pro tyr val

2582                                                    2612
GGA GCC GAC TTG AAA GTC ATG GAA AGG GAT TCT AAA AAA ATC GCG GCG AAA AGC CCT TTA
gly ala asp leu lys val met glu arg asp ser lys lys ile ala ala lys ser pro leu

2642                                                    2672
TTT TTA CCG AAT ATC CGC GCT AAA GAA GGT TTA GAC GAT GTG ATC GCT TGG ATC AAG CGC
phe leu pro asn ile arg ala lys glu gly leu asp asp val ile ala trp ile lys arg

2702
AAC GCT TTA TTG GAA GAT TGA TGA ACA CTT
asn ala leu leu glu asp OPA

2701                    SD                              2731
CAA CGC TTT ATT GGA AGA TTG ATG AAC ACT TAC GCT CAA GAA TCC AAG CTC AGG TTA AAA
       ureH                    Met asn thr tyr ala gln glu ser lys leu arg leu lys

2761                                                    2791
ACC AAA ATA GGG GCT GAC GGG CGG TGC GTG ATT GAA GAC AAT TTT TTC ACG CCC CCC TTT
thr lys ile gly ala asp gly arg cys val ile glu asp asn phe phe thr pro pro phe

2821                                                    2851
AAG CTC ATG GCG CCC TTT TAC CCT AAA GAC GAT TTA GCG GAA ATC ATG CTT TTA GCG GTA
lys leu met ala pro phe tyr pro lys asp asp leu ala glu ile met leu leu ala val
```

FIGURE 4 (cont.)

2881 2911

AGC CCT GGC TTA ATG AAA GGC GAT GCA CAA GAT GTG CAA TTG AAC ATC GGT CCA AAT TGC

ser pro gly leu met lys gly asp ala gln asp val gln leu asn ile gly pro asn cys

2941 2971

AAG TTA AGG ATC ACT TCG CAA TCC TTT GAA AAA ATC CAT AAC ACT GAA GAC GGG TTT GCT

lys leu arg ile thr ser gln ser phe glu lys ile his asn thr glu asp gly phe ala

3001 3031

AGC AGA GAC ATG CAT ATC GTT GTG GGG GAA AAC GCT TTT TTA GAC TTC GCG CCC TTC CCG

ser arg asp met his ile val val gly glu asn ala phe leu asp phe ala pro phe pro

3061 3091

TTA ATC CCC TTT GAA AAC GCG CAT TTT AAG GGC AAT ACC ACG ATT TCT TTG CGC TCT AGC

leu ile pro phe glu asn ala his phe lys gly asn thr thr ile ser leu arg ser ser

3121 3151

TCC CAA TTG CTC TAT AGT GAA ATC ATT GTC GCA GGG CGA GTG GCG CGC AAT GAG TTG TTT

ser gln leu leu tyr ser glu ile ile val ala gly arg val ala arg asn glu leu phe

3181 3211

AAA TTC AAC CGC TTG CAC ACC AAA ATC TCT ATT TTA CAA GAT GAG AAA CCC ATC TAT TAT

lys phe asn arg leu his thr lys ile ser ile leu gln asp glu lys pro ile tyr tyr

3241 3271

GAC AAC ACG ATT TTA GAT CCC AAA ACC ACC GAC TTA AAT AAC ATG TGC ATG TTT GAT GGC

asp asn thr ile leu asp pro lys thr thr asp leu asn asn met cys met phe asp gly

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FIGURE 4 (cont.)

```
3301                                                    3331
TAT ACG CAT TAT TTG AAT TTG GTG CTG GTC AAT TGC CCC ATA GAG CTG TCT GGC GTG CGA
tyr thr his tyr leu asn leu val leu val asn cys pro ile glu leu ser gly val arg

3361                                                    3391
GGA TTG ATT GAA GAG AGC GAA GGA GTG GAT GGA GCC GTG AGT GAA ATC GCT AGT TCT CAT
gly leu ile glu glu ser glu gly val asp gly ala val ser glu ile ala ser ser his

3421                                                    3451
TTA TGC CTG AAA GCT TTA GCG AAA GGC TCA GAA CCC TTG TTG CAT TTA AGA GAA AAA ATC
leu cys leu lys ala leu ala lys gly ser glu pro leu leu his leu arg glu lys ile

3481                                                    3511
GCT CGC TTT ATC ACG CAA ACG ATT ACG CCA AAG GTT TAA AAA ACA CTT TAA AAA AGA TTA
ala arg phe ile thr gln thr ile thr pro lys val OCH

3541
TAC CCT TTA GTC TTT TTT AA
```

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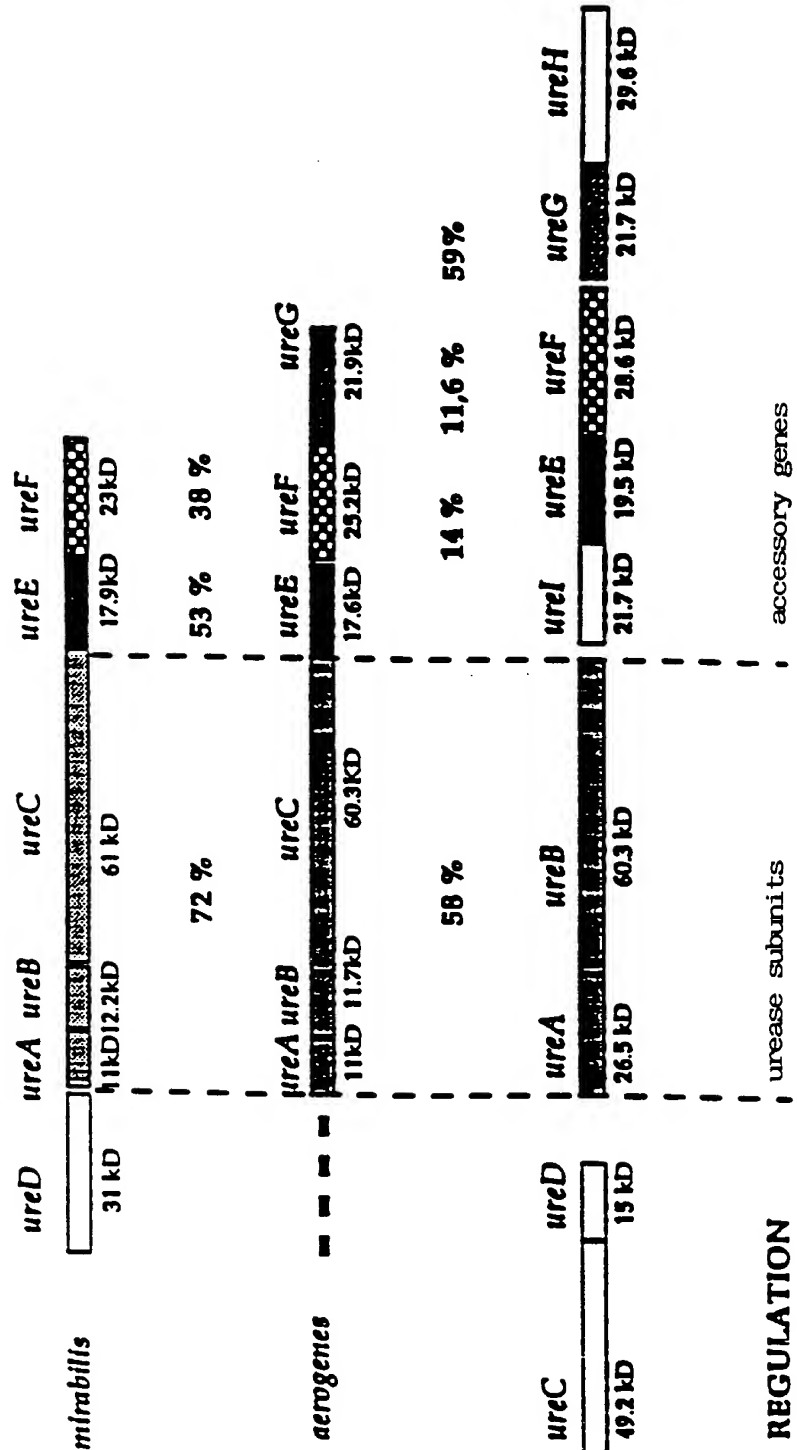
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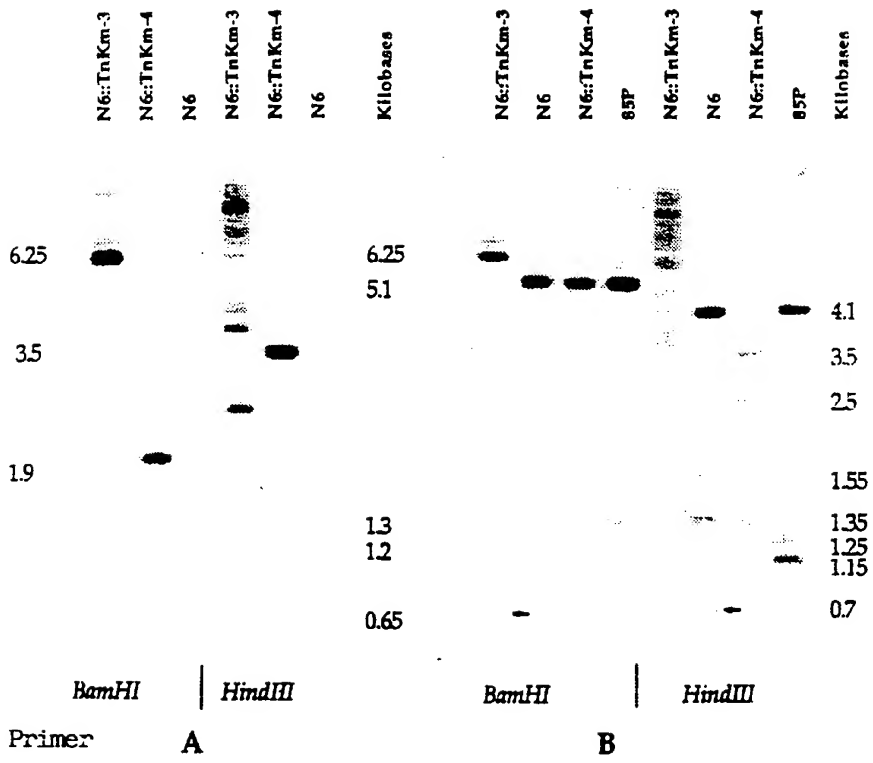
ureA
ureB
ureC
ureE
ureF
ureG
ureH
ureI
Proteus mirabilis
31 kD
11kD
12.2kD
61 kD
17.9kD
23kD
72 %
53 %
38 %
Klebsiella aerogenes
11kD
11.7kD
60.3KD
17.6kD
25.2kD
21.9kD
58 %
14 %
11,6 %
59%
H. pylori
26.5 kD
60.3 kD
21.7 kD
19.5 kD
28.6 kD
21.7 kD
29.6 kD
49.2 kD
15 kD
REGULATION
urease subunits
accessory genes

FIGURE 5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N6::TnKm-4
N6
N6::TnKm-3
N6::TnKm-4
N6
Kilobases
N6::TnKm-3
N6
N6::TnKm-4
85P
N6::TnKm-3
N6
N6::TnKm-4
85P
Kilobases
6.25
3.5
1.9
6.25
5.1
1.3
1.2
0.65
4.1
3.5
2.5
1.55
1.35
1.25
1.15
0.7
BamHI
HindIII
BamHI
HindIII
Primer
A
B

FIG. 6

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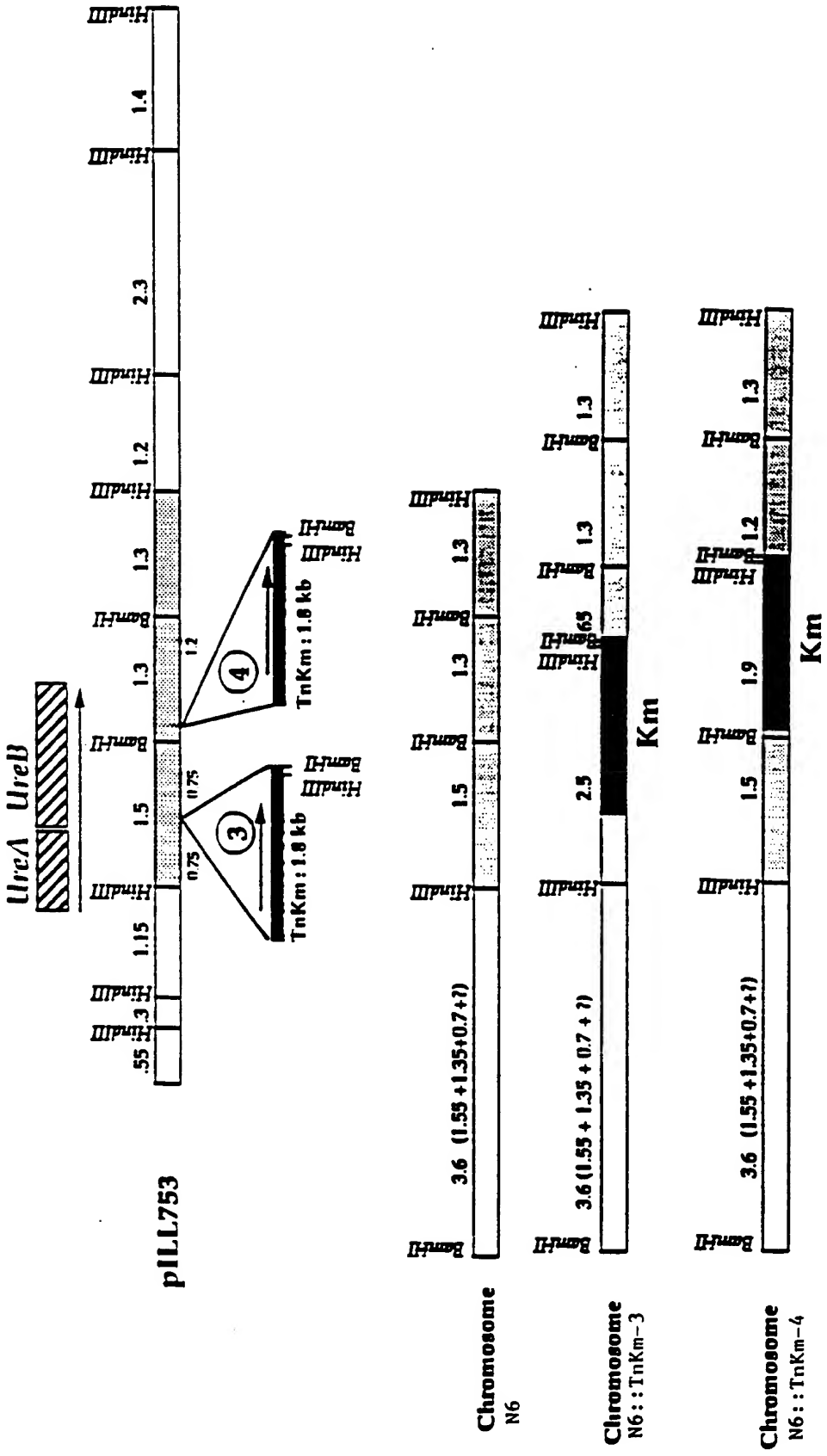

FIGURE 7

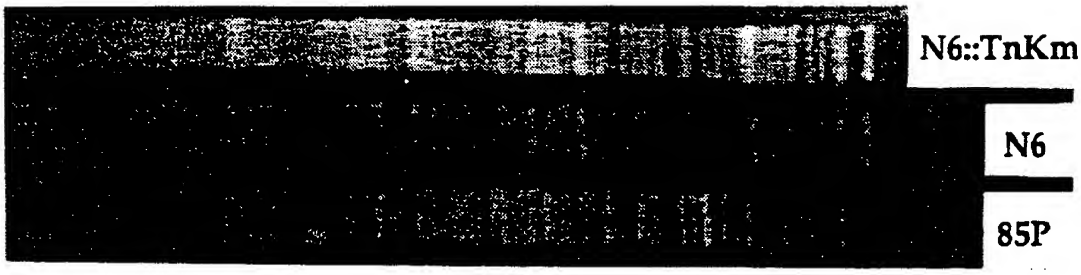

FIGURE 8

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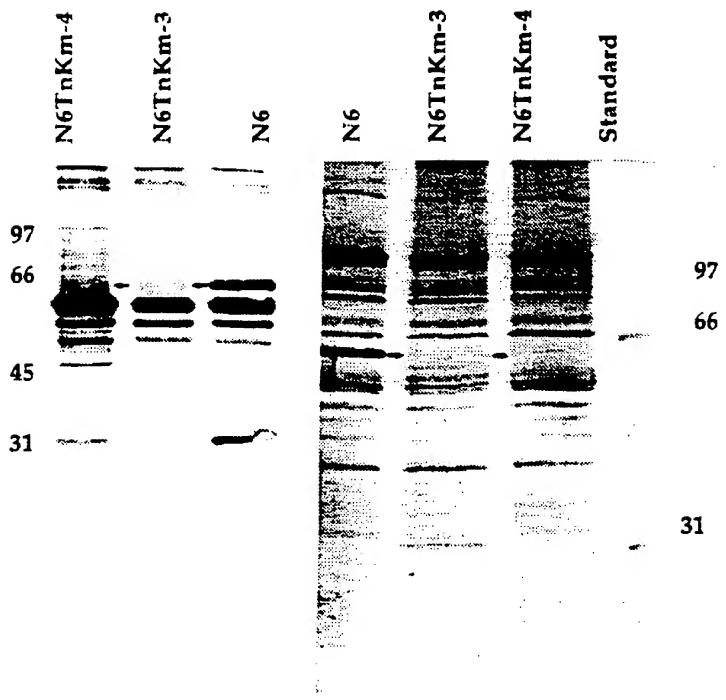
FIGURE 9

FIGURE 10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N6TnKm-3
N6
N6
N6TnKm-3
N6TnKm-4
Standard
97
66
45
31
97
66
31
Anti-urease diluted 1:1,000
Human anti-serum diluted 1:1,000

FIGURE 11

FIGURE 12

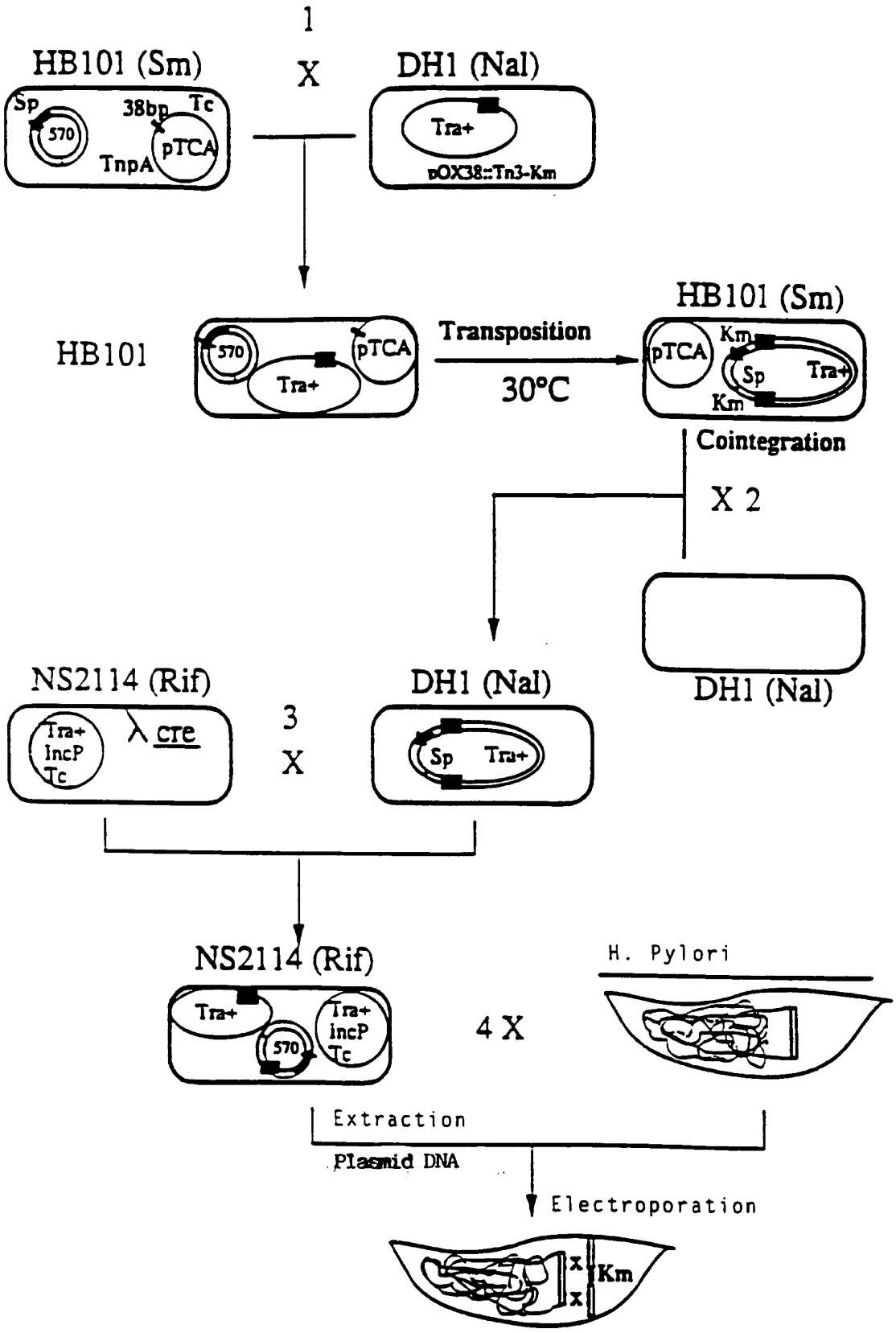
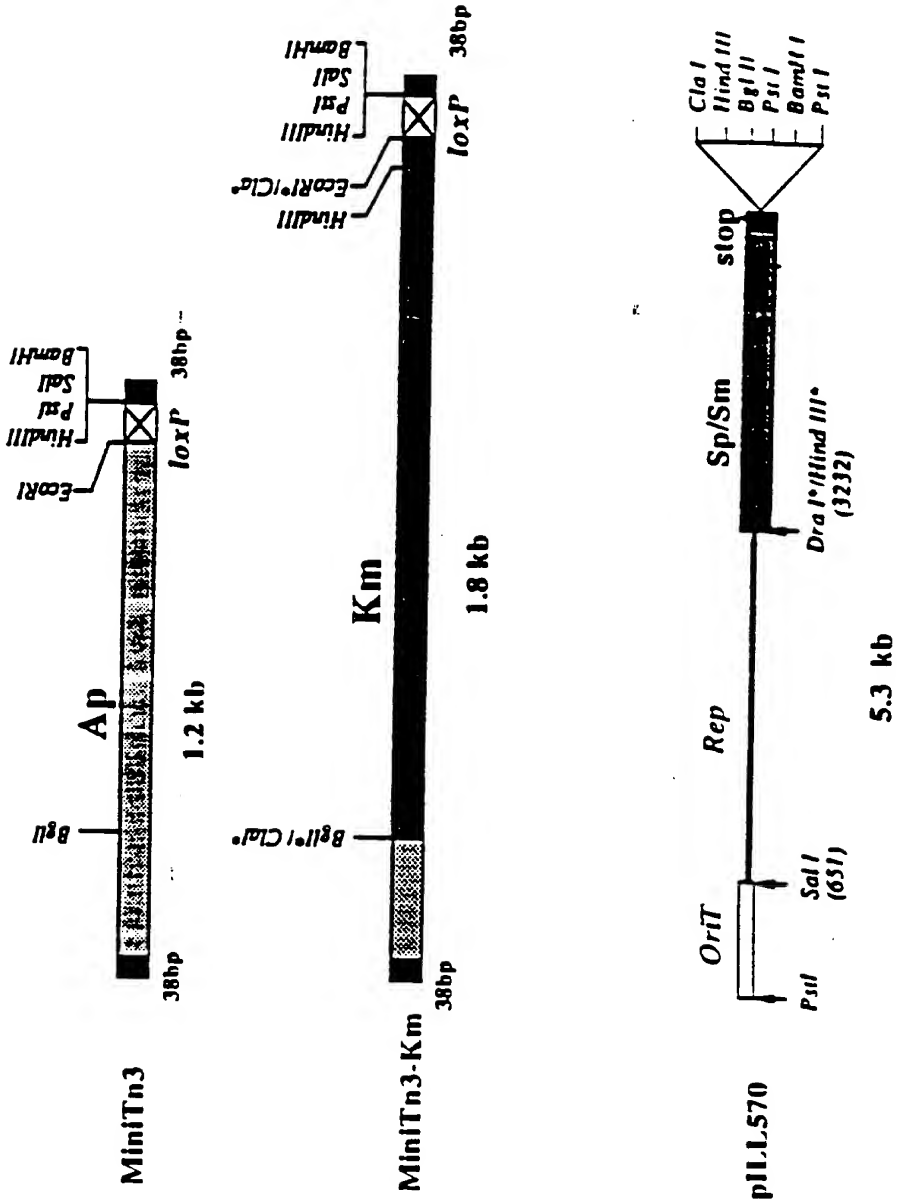

1
HB101 (Sm)
X
DH1 (Nal)
Sp
38bp
Tc
570
TnpA
pTCA
Tra+
pOX38::Tn3-Km
HB101
570
pTCA
Tra+
Transposition
30°C
HB101 (Sm)
pTCA
Km
Sp
Tra+
Km
Cointegration
X 2
DH1 (Nal)
NS2114 (Rif)
Tra+
IncP
Tc
λ cre
3
X
DH1 (Nal)
Sp
Tra+
NS2114 (Rif)
Tra+
570
Tra+
IncP
Tc
4 X
H. Pylori
Extraction
Plasmid DNA
Electroporation
X
X
Km

FIGURE 13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38bp
BglI
EcoRI
HindIII
PstI
SalI
BamHI
loxP
MiniTn3-Km
Km
1.8 kb
BglII* ClaI*
EcoRI*ClaI*
pIL570
Rep
5.3 kb
OriT
PstI
SalI (651)
Sp/Sm
stop
DraI*HindIII* (3232)
ClaI
HindIII
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